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Analysis of Pesticide Raid® in Feed of Wistar Rat by High-Pressure Liquid Chromatography (HPLC)

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Abstract

The distribution of pesticide by-product in tissues of wistar rats were analyzed using high pressure liquid chromatography. The limit of detection of the HPLC was 0.1 µg. Results show bioaccumulation factor of pesticide “Raid®” in lipid, up to three times that of the feed at the first concentration and gradually decreased as the concentration increased in the muscle > (0.7), brain > (0.5) and liver > (0.3) as indicated in the text. At higher concentration of 961 µg/g, bioaccumulation factor decreased in the lipid to 1.2 and 0.6 in the muscle, 0.03 in the brain and 0.08 in the liver respectively. High Pressure Liquid Chromatography (HPLC) analysis of raid extract suggests the presence of micprothrin and palethrin. The implications are numerous, but simply put that accidental ingestion of chlorinated hydrocarbon as in “Raid®” may involve convulsions, collapse and coma after only brief excitation and ataxia at the onset.

Keywords: High Pressure Liquid Chromatography, Pesticide, Raid®, Chlorinated Hydrocarbon, Bioaccumulation

1. Introduction

Pesticides have been used to boost food production to a considerable extent and to control vectors of disease [1]. However, these advantages of great economic benefits sometimes come with disadvantages when subjected to critical environmental and human health considerations. The chronic effects of pesticides from food intake on human health are not well defined, but there is increasing evidence of carcinogenetic [2], teratogenic [3] and genotoxic [4], as well as disruption of hormonal functions [5]. Analysis of these pesticides and their residues had in the past aided objective re-evaluation and reassessment of these substances on a benefit-risk analysis basis and their subsequent withdrawal from use when found to be hazardous to human health and the environment.

Residues of pesticides and heavy metals enter the food system. This occurs more often than one might think. Additionally, residues of pesticides found in the air inside the home and on floors and other interior surfaces contribute to non-occupational pesticide exposure [6]. For example, any potentially harmful substances that have no set tolerance thresh may not necessarily be harmful. This means that if there is any toxin in food supply or water, such food cannot be ban unless it exists

in quantities over the tolerance threshold no matter what. There are few studies of compliance to pesticide use [7,8].

The quality and sophistication of analysis have grown and very minute quantities of these pesticides and their residues can be analyzed with high degree of specify, precision and accuracy. Therefore, it is important and utmost necessary to develop methods for analysis of pesticides residues in environmental samples. In the light of the above, and in view of pesticide related adverse reactions, the study, presents chromatographic and absorption spectra from a common pesticide Raid® in tissue of rat as it affects basal metabolism using high pressure liquid chromatograph.

2. Materials and Methodology

2.1. Test Facility

Tests with Wistar rats were conducted in a room temperature exposure facility (23°C). Thirty-five animals weighing between 180 - 210 were caged in perforated aluminum chambers (38 × 55 × 35 cm). Five animals were placed in each of the chambers containing sawdust shaves, and a light: dark cycle of approximately 12 h.

Water was given ad libitum, temperature was maintained at $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Air temperature was $\pm 2^{\circ}\text{C}$ of the water temperature. Protocols describing the use of animals in accordance with National Research Council (NRC) [8] and World Medical Association (WMA) [9] guide on the care and use of laboratory animals.

2.2. Test procedures

Wistar rats were obtained from the animal breeding facility of College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Technical grade "RAID" (Johnson Wax, Nigeria), was dissolved in corn oil and with (in a rotary mixer) 2:98 (w/w) the commercial feed, Purina 5000, for 10 min: the control diet contained corn oil in the same proportion. Animals were fed raid-spiked commercial feed at measured concentrations of 25.0 ± 2.4 , 54.0 ± 9.2 , 108.0 ± 12.5 , 216.0 ± 14.6 , and 430.0 ± 20.2 and 961.0 ± 80.6 $\mu\text{g/g}$ "Raid" for 10 days, increasing 5 - 10 g/day and ending with 96 g/rat. Two rats from different chambers receiving the same concentration of raid in the food were selected and tissue samples were dissected out (lipid, muscle, brain and liver) at test termination. The symptom-limited sensation tests were performed after an overnight fast [10]. Mean values were calculated from the two rats sampled from each concentration of insecticide-raid.

2.3. Test Extraction

In the toxicity persistence test, "Raid" was extracted with 1:1 diethyl ether-petroleum ether [11]. This compound was identified and quantified by high-pressure liquid chromatography (HPLC), using UV1-202nm@01, Base (Amersham Pharmacia Biotech), serial number 90042, mobile phase was Methanol: H_2O (1:1). The limit of detection of the HPLC was 0.1 μg . The dimension of the column C18, 4.6×250 mm and particle size 5 micron. Flow rate was 1 ml/min, the injection volume 100 μl and a wavelength of 202 nm was used for detection. The values were quantified by standard calibration curve.

2.4. Analytical Procedures

The brain, muscle and liver homogenate fractions were prepared by the method described by [12] and resuspended in 0.15 M KCl to an appropriate final concentration of 20 mg of protein per millimeter (approximately) 0.5 g of tissue. Liver tissue lipids were extracted by chloroform/methanol ratio (2:1) following the method of Folch [13]. Fractions of each tissue homogenate were used for the determination of Glucose-6-phosphatase (G-6-Pase) and Lactate dehydrogenase respectively, to

test for inhibition of metabolic enzymes required to aid digestion. Glucose-6-phosphatase was determined using aliquot of each homogenate (20%), incubated with 1 ml of P-nitrophenyl phosphate (NPP) for 15 min and the reaction was stopped by addition of 2 ml of 0.2 N NaOH; absorbance was measured at 450 nm as described by [14]. Lactate dehydrogenase was determined by the method of [15]. Protein was determined by [16] method. Experiments were conducted in a completely randomized manner with five replicates. These were repeated and mean data of two experiments is presented. Data were subjected to one-way analysis of variance and significance of treatments from control was tested at 5% level of significance followed by Dunnet's test. Further, data were also subjected to determination of correlation coefficient between raid concentration and the observed response. The statistical analysis was performed using SPSS software version 10.0.

3. Results and Discussion

Bioaccumulation factor of pesticide "Raid®" was observed in lipid, up to three times that of the feed at the first concentration and gradually decreased as the concentration increased (**Table 1**) while accumulation factor in the muscle (0.7), brain (0.5) and liver (0.3) was about the indicated number times that of the feed. At higher concentration of 961 $\mu\text{g/g}$, bioaccumulation factor decreased in the lipid to 1.2 and 0.6 in the muscle, 0.03 in the brain and 0.08 in the liver. Using the mean of insecticide in feed, the tissues accumulate the insecticide in ascending order: brain < liver < muscle < lipid. The effects increased with increasing insecticide concentration in the feed and showed a significant deference compared to control ($P < 0.05$) at a concentration of 430 $\mu\text{g/g}$.

Table 2 indicates the estimated detectable levels of toxicity in rat tissues exposed to the insecticide "Raid®". Tissue insecticide concentration increased in lipid, muscle, brain and liver, respectively. The insecticide accumulation in these organs may therefore indicate that the active ingredients are also absorbed resulting in the decreased enzymes formation, which should accelerate immediate metabolic processes, but instead, remained complexed in the tissue organs. Additionally, the retention time (within 10 min.) may be increased because of its easy solubility and lower excretion rate leading to bioaccumulation. The brain shows insignificant decreases in the enzymes Glucose-6-phosphatase and Lactic acid dehydrogenase, while significant decreases were noticeable in the muscle and liver. The tissues concentrations were the sums of the two components.

High Pressure Liquid Chromatography (HPLC) analysis of raid extract suggests the presence of micprothrin

Table 1. Tissue total raid concentrations and bioaccumulation factors (BAF) in Wister Rats exposed to insecticide “Raid”.

Mean $\pm$ SD insecticide “Raid” in feed ($\mu\text{g/g}$)	Raid concentration in Wister rats ($\mu\text{g/g}$) ^a and bioaccumulation factor (BAF) ^b			
	Lipid	Muscle	Brain	Liver
0.00	-	-	-	-
25.0 $\pm$ 2.4	72.5 (2.9)	17.5 (0.7)	12.5 (0.5)	7.5 (0.3)
54.0 $\pm$ 9.2	86.4 (1.6)	21.7 (0.4)	16.4 (0.3)	9.4 (0.2)
108.2 $\pm$ 12.5	172.8 (1.6)	30.4 (0.3)	19.5 (0.2)	10.8 (0.10)
216.2 $\pm$ 14.6	280.8 (1.3)	45.8 (0.2)	22.9 (0.1)	19.8 (0.09)
430.0 $\pm$ 20.6	324.0 (0.8)	86.4 (0.2)	25.8 (0.06)	37.3 (0.09)
961.2 $\pm$ 70.5	1153.2 (1.2)	576.6 (0.6)	28.8 (0.03)	76.9 (0.08)

^aMean raid concentration at test termination (~steady state value); ^bBAF = bioaccumulation factor ‘Raid’ in tissue ($\mu\text{g/g}$)/feed raid concentration ($\mu\text{g/g}$).

Table 2. Index of toxicity in rat tissues exposed to insecticide “Raid”.

Indicators	Brain IU/L	Muscle IU/L	Liver IU/L
Control	0.28 $\pm$ 0.02	1.15 $\pm$ 0.20	2.54 $\pm$ 0.1
Glucose-6-phosphatase	0.25 $\pm$ 0.05	0.35 $\pm$ 0.09*	0.15 $\pm$ 0.03*
Control	0.22 $\pm$ 0.04	0.10 $\pm$ 0.01	1.94 $\pm$ 0.02
Lactate dehydrogenase	0.19 $\pm$ 0.08	0.15 $\pm$ 0.11*	0.07 $\pm$ 0.08*

*Significantly different $P < 0.05$ from control.

and palethrin. **Figure 1(a)** shows the chromatographic profile of the raid insecticides. **Figure 1(b)** shows the chromatographic profile of standards (0.025%). The highest peak obtained from “Raid®” extract is most likely microthrin. This peak possessed a retention time (9.25) min. For the identification of raid, co-chromatography with authentic standards in different chromatographic conditions was used. Two different peaks were eluted within 10 min and were well resolved (**Figure 1(a)**), but unidentified peak was eluted within a retention time of early eluting of 2 min. The HPLC analysis failed to detect the unidentified component in “Raid®”. The reason is not clear, but raid may have undergone a structural change because of combination, hence different absorbance property [17]. The fine tuning of the mobile phase composition depended on the choice of the internal standard. Most internal standard compounds were rejected because of interfering components. Only microthrin and

palethrin had acceptable chromatographic characteristics. The implications are numerous, but simply put that accidental ingestion of chlorinated hydrocarbon as in “Raid®” may involve convulsions, collapse and coma after only brief excitation and ataxia at the onset.

Other workers have contrasted neuritic outgrowth of chlorpyrifos, diazinon and organophosphate pesticides including parathion and concluded that all have adverse effects on brain development and systemic toxicity [18]. In recent study, Walz, [19], shows that glyphosate, the active ingredient in Roundup, causes birth defects in frogs and chicken embryos at far lower levels than used in agricultural and garden applications. The malformations primarily affected the skull, face, midline and spinal cord. Other independent scientific research has also found that glyphosate causes endocrine disruption, developmental and reproductive toxicity, DNA damage, neurotoxicity and cancer [3,18]. Many of these effects

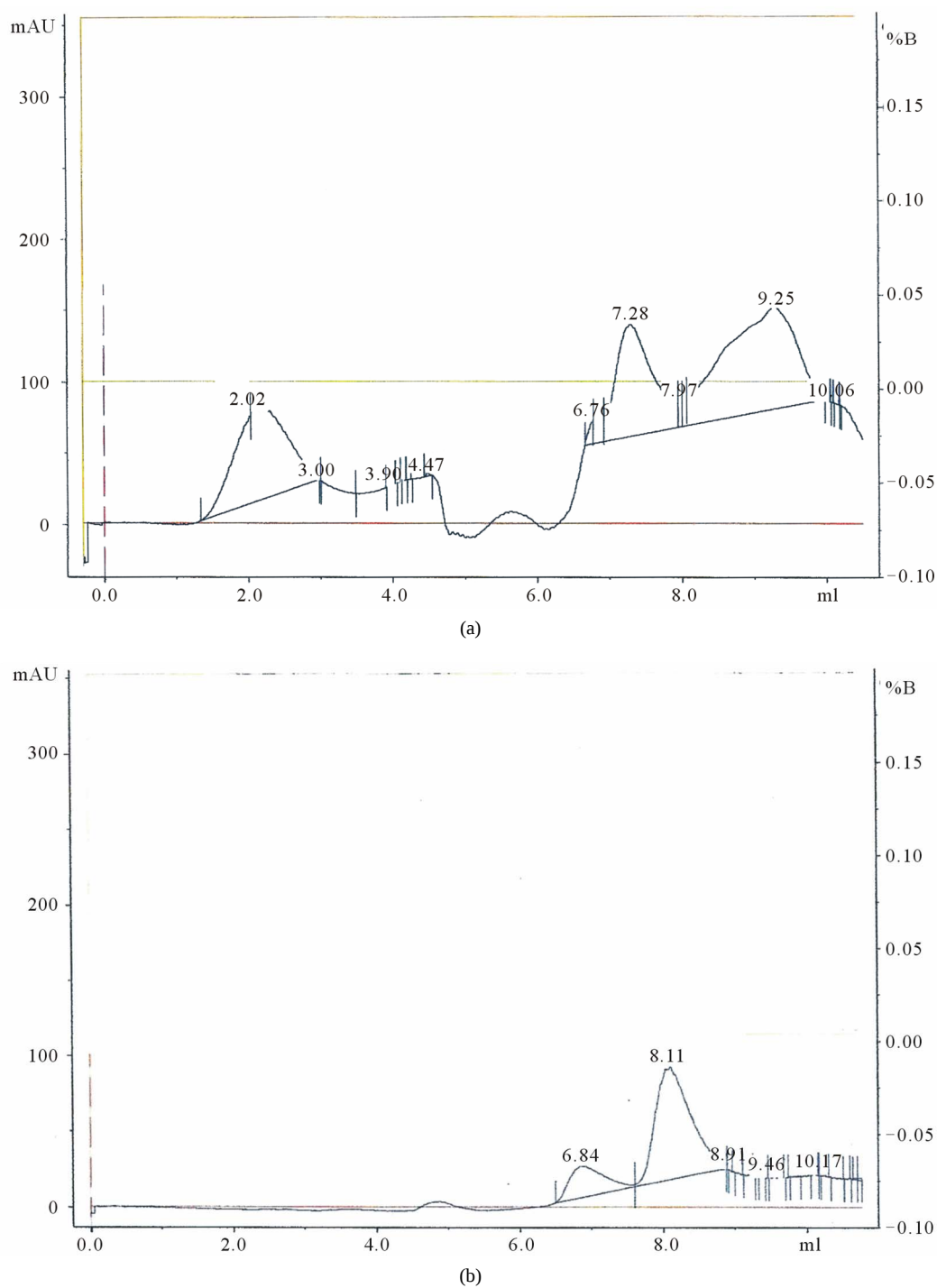


Figure 1. Chromatograms and absorption spectra from analysis of "Raid" extract in tissue of rat. Analysis of microprothin and palethrin (a) and standard peak (b).

were apparent at much lower doses than the typical levels of pesticide residues found in food [20,21]. Yet despite the evidence [which strongly support the National Campaign for Sustainable Agriculture, (a diverse partnership of individuals and organizations), cultivating grass

roots efforts to engage in policy development processes that may result in food, agricultural systems and rural communities that are healthy, environmentally sound, profitable, humane and just [22], pesticides continue to be found in agricultural produce and lurking in tap water.

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